Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembly

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Abstract Recently exonic and intronic mutations in the gene for microtubule-associated protein tau have been discovered in cases of familial frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Intronic mutations have been shown to lead to an abnormal preponderance of four-repeat tau isoforms. The effects of the exonic mutations are unknown. We report here that the G272V, P301L, V337M and R406W mutations lead to a marked reduction in the ability of tau to promote microtubule assembly. This partial loss-of-function may be the primary effect of the known missense mutations in tau.

Key words: Tau protein; Microtubule assembly; Frontotemporal dementia; Alzheimer's disease

1. Introduction

Microtubule-associated protein tau is the major component of the filamentous neurofibrillary lesions of Alzheimer's disease and other tauopathies [1]. The mechanisms that lead to the formation of hyperphosphorylated tau filaments in brain are only poorly understood. Recently, exonic and intronic mutations in the tau gene have been identified in familial 'frontotemporal dementia and parkinsonism linked to chromosome 17' (FTDP-17) [2–4]. Four separate missense mutations and four separate intronic mutations have been described in 13 families with FTDP-17.

In adult human brain, six tau isoforms are produced from a single gene by alternative mRNA splicing [5]. They differ by the presence or absence of inserts of 29 and 58 amino acids located in the amino-terminal half and a 31 amino acid repeat located in the carboxy-terminal half. Inclusion of the latter, which is encoded by exon 10 of the tau gene [5–7], gives rise to tau isoforms with four microtubule-binding repeats. Intronic mutations are located close to the splice-donor site of the intron following exon 10, where they disrupt a predicted stem-loop structure [3,4]. This has been shown to lead to increased levels of the three tau isoforms with four microtubule-binding repeats and to reduced levels of the three isoforms with three microtubule-binding repeats [3,4]. Overproduction of four-repeat tau isoforms may lead to an excess of tau over available binding sites on microtubules [4], resulting in the assembly of four-repeat isoforms into wide twisted ribbon-like filaments [8]. The known exonic mutations in tau are located in the microtubule-binding repeat region or close to it, suggesting that they may interfere with the ability of tau to interact with microtubules [2,3]. We have therefore examined the effects of these mutations in the context of a three-repeat and a four-repeat tau isoform on the promotion of microtubule assembly.

2. Materials and methods

2.1. Expression and purification of wild-type and mutated tau proteins

Site-directed mutagenesis was used to change G272 to valine, V337 to methionine and R406 to tryptophan in the three-repeat 381 amino acid isoform of human tau (expressed from cDNA clone htau37) and in the four-repeat 412 amino acid isoform (expressed from cDNA clone htau46) (in the numbering of the 441 amino acid isoform of human tau). P301 was changed to leucine in the four-repeat isoform. All constructs were verified by DNA sequencing. Wild-type and mutated tau proteins were expressed in *E. coli* BL21(DE3), as described [9]. Bacterial pellets were resuspended in 50 mM PIPES, 1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 0.5 μg/ml leupeptin, pH 6.8, followed by a 2×1-min sonication on ice using a Kontes Micro Ultrasonic Cell Disrupter. The homogenates were centrifuged at 27 000×*g* for 15 min and the supernatants filtered through a 0.45-μm Acrodisc. The filtrate was loaded onto a phosphocellulose column (bed volume 2 ml) equilibrated in extraction buffer. The column was washed in extraction buffer, followed by extraction buffer+0.1 M NaCl. Protein was eluted batchwise with 6 ml extraction buffer containing 0.3 M NaCl. This was followed by overnight dialysis against a saturated ammonium sulfate solution and precipitation by a 10-min centrifugation at 50 000 rpm (Beckman TL100). The pellet was resuspended in extraction buffer and reprecipitated by addition of an equal volume of saturated ammonium sulfate. Following centrifugation, the pellet was resuspended in 1 ml extraction buffer containing 0.5 M NaCl and 1% 2-mercaptoethanol, and boiled for 3 min. Following a 10-min centrifugation at 50 000 rpm, the supernatant was loaded onto a NAP10 column equilibrated in 80 mM PIPES, 1 mM EGTA, 0.2 mM $MgCl_2$, 1 mM DTT (microtubule assembly buffer minus GTP) and eluted with 1.5 ml of the same buffer. GTP was added to 1 mM. Tau protein concentrations were determined using densitometry (Molecular Dynamics) and calibration against tau protein of known concentration. Bovine serum albumin was used as the standard for densitometry. In all experiments, wild-type and mutant tau proteins were expressed and purified in parallel.

2.2. Microtubule assembly

Four-repeat tau isoforms assemble microtubules at a 2.5–3.0-fold faster rate than isoforms with three repeats [9]. To obtain similar rates of assembly, we used a higher concentration of three-repeat than of four-repeat tau isoforms. Purified recombinant wild-type and mutated htau37 (0.3 mg/ml, 6.9 μM) and htau46 (0.1 mg/ml, 2.3 μM) proteins were incubated with bovine brain tubulin (1 mg/ml, 20 μM, Cytoskeleton) in assembly buffer at 37°C, as described [10]. The assembly of tubulin into microtubules was monitored over time by a change in turbidity at 350 nm.

3. Results

Tau proteins with mutations showed a reduced ability to promote microtubule assembly (Figs. 1 and 2). The smallest effect was observed with the R406W mutant and the largest effect with the P301L mutant. The V337M and G272V mutations produced intermediate effects. All the mutations had a

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larger effect in three-repeat than in four-repeat tau isoforms. Thus, the R406W mutation led to a 40% reduction in the rate of microtubule assembly in the three-repeat tau isoform, but only to a 20% reduction in the four-repeat isoform (expressed as the optical density at 2 min). An 85% reduction was found for the V337M mutation in the three-repeat tau isoform and a 55% reduction in the four-repeat isoform. The G272V mutation produced a 70% reduction in the rate of microtubule

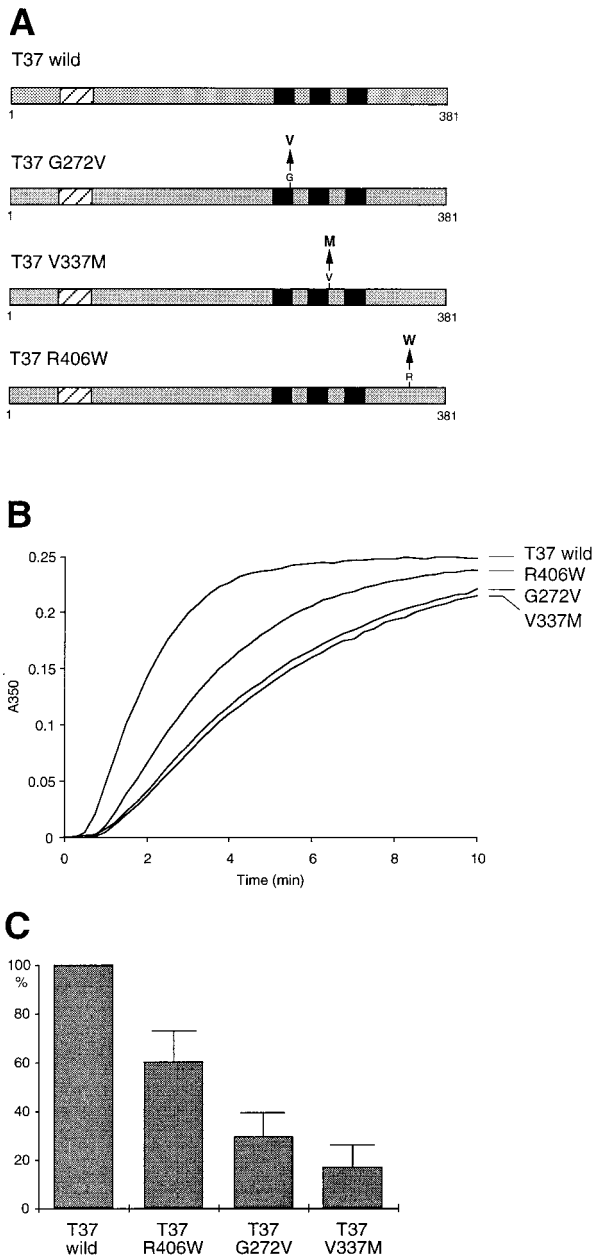

Fig. 1. Effects of missense mutations in tau on the ability of three-repeat htau37 (381 amino acid isoform of human tau) to promote microtubule assembly. A: Schematic diagram of wild-type and mutated htau37. The three tandem repeats are shown as black bars, with the alternatively spliced exon 2 shown as shaded bar. The positions of the FTDP-17 mutations at residues 272, 337 and 406 are indicated. B: Polymerisation of tubulin induced by wild-type htau37, htau37 G272V, htau37 V337M and htau37 R406W, as monitored over time by turbidimetry. A typical experiment is shown; similar results were obtained in three separate experiments. C: Optical densities for wild-type htau37 and the three mutants at 2 min (expressed as % of wild-type htau37, taken as 100%). The results are expressed as means ± S.E.M. ($n = 3$). Amino acid numbering is according to the 441 amino acid isoform of human tau.

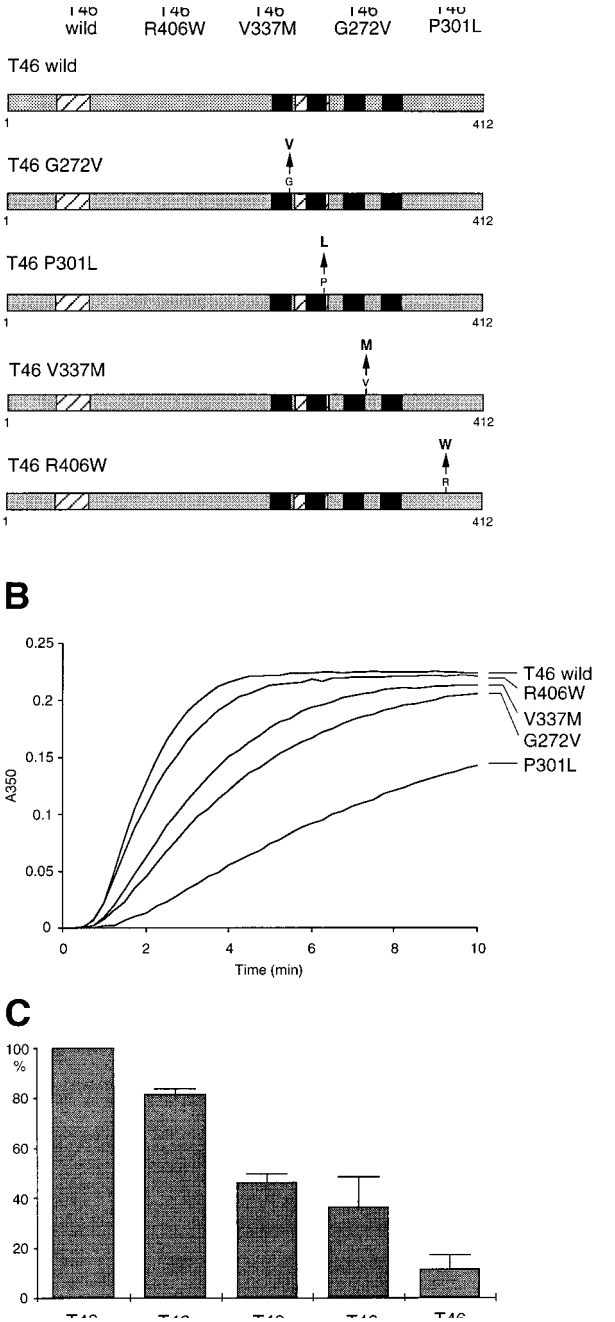

Fig. 2. Effects of missense mutations in tau on the ability of four-repeat htau46 (412 amino acid isoform of human tau) to promote microtubule assembly. A: Schematic diagram of wild-type and mutated htau46. The four tandem repeats are shown as black bars, with the alternatively spliced exons 2 and 10 shown as shaded bars. The positions of the FTDP-17 mutations at residues 272, 301, 337 and 406 are indicated. B: Polymerisation of tubulin induced by wild-type htau46, htau46 G272V, htau46 P301L, htau46 V337M and htau46 R406W, as monitored over time by turbidimetry. A typical experiment is shown; similar results were obtained in three separate experiments. C: Optical densities for wild-type htau46 and the four mutants at 2 min (expressed as % of wild-type htau46, taken as 100%). The results are expressed as means ± S.E.M. ($n = 3$). Amino acid numbering is according to the 441 amino acid isoform of human tau.

assembly in the three-repeat tau isoform and a 60% reduction in the four-repeat isoform. The P301L mutation, which is only found in four-repeat tau isoforms, produced a 90% reduction in the rate of tubulin polymerisation (Fig. 2).

4. Discussion

These findings demonstrate that missense mutations in tau which are associated with FTDP-17 lead to a reduction in the ability of tau protein to promote microtubule assembly, akin to a partial loss-of-function. Microtubule assembly as measured in this assay is the product of microtubule nucleation and growth. Future experiments will separately test the effects of the tau mutations on microtubule nucleation and growth. The three-repeat tau isoform was affected more strongly by the mutations than the four-repeat isoform, indicating a differential isoform effect of the G272V, V337M and R406W mutations. The effects of the G272V and V337M mutations were stronger than those of the R406W mutation, in keeping with their location in the microtubule-binding repeat region. The R406W mutation is located downstream of the repeats. The P301L mutation produced the largest reduction in the rate of microtubule assembly. It is located in the alternatively spliced repeat which gives rise to tau isoforms with four repeats. Unlike the G272V, V377M and R406W mutations which affect all six tau isoforms, the P301L mutation only affects four-repeat isoforms.

It is well established that the repeat region of tau and some flanking sequences are required for full biological activity [11]. However, relatively little is known about the exact sequence requirements. Several positively charged residues have been shown to have a strong influence on the ability of tau to promote microtubule assembly [12–14], in keeping with evidence indicating that the binding of tau to microtubules is governed by electrostatic interactions [15]. The present results show that the sequence requirements in tau for the promotion of microtubule assembly are not limited to charge interactions, since of the four missense mutations only the R406W mutation leads to a reduction in positive charge. They are consistent with findings showing that the nucleation of microtubules involves steps that are not affected by the binding of tau to microtubules [16,17] and suggest that the known exonic mutations in tau may affect the nucleation of microtubules.

The partial loss-of-function resulting from the reduced ability of mutated tau to promote microtubule assembly is probably the primary effect of the tau mutations. It may represent a necessary step for permitting the subsequent hyperphosphorylation of tau which, in conjunction with other factors [10,18–20], is believed to lead to assembly into filaments. It remains to be determined whether the mutations have additional effects on tau phosphorylation and filament assembly.

Tau mutations in the intron following exon 10 or in exon 10 itself lead to a neuronal and glial pathology [4,21], whereas mutations located outside exon 10 lead to a predominantly neuronal tau pathology [21–23]. In normal adult human brain, tau is predominantly found in nerve cells, with only low levels in glial cells [6,24–27]. Six tau isoforms are expressed, with a slight preponderance of three-repeat over four-repeat isoforms [5,9]. Although it is not known which tau isoforms are expressed in glial cells, the cellular pathology of FTDP-17 is compatible with nerve cells expressing all six tau isoforms and glial cells expressing predominantly four-repeat isoforms. In the case of mutations located outside exon 10, the ordered assembly of tau into filaments may be driven by three-repeat isoforms, consistent with the larger effects of mutated three-repeat tau reported here.

The sequence of events, which leads from a reduced ability of tau to interact with microtubules to assembly into filaments, may also apply to other diseases with a filamentous tau pathology, such as Alzheimer's disease, progressive supranuclear palsy, Pick's disease and corticobasal degeneration. Thus, the V337M mutation in tau leads to a tau pathology which is indistinguishable from that of Alzheimer's disease in its ultrastructural and biochemical characteristics [2,22]. The R406W mutation gives rise to a tau pathology with some characteristics reminiscent of Alzheimer's disease [3,23]. The G272V mutation in tau leads to the formation of Pick-like bodies, whereas the P301L mutation produces a tau pathology similar to that of corticobasal degeneration and progressive supranuclear palsy [3,21].

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